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Water Stress Reduces Ozone Injury via a Stomatal Mechanism

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ABSTRACT

Various studies have shown that water-stressed plants are more tolerant of ozone exposures than are unstressed plants. Two probable explanations for this tolerance are (a) stomatal closure which reduces ozone uptake and (b) biochemical or anatomical changes within the leaves. *Phaseolus vulgaris* cv Pinto bean plants were established and transferred to membrane systems which controlled the osmotic potential around the roots at -35 or -80 kilopascals for 5 days prior to ozone treatment (0 or 1.0 microliters per liter for 2 hours). Both water-stressed and unstressed plants were sprayed with various concentrations of abscisic acid to close the stomata or with fusicoccin to induce stomatal opening. The abaxial stomatal resistances of primary and trifoliate leaves were measured just prior to ozone exposure. Plant response to ozone was determined by stress ethylene production and chlorophyll loss. Both water stress and abscisic acid induced stomatal closure and reduced ozone injury. In water-stressed plants, fusicoccin induced stomatal opening and those plants were as sensitive to ozone as were the non-water-stressed plants. These data suggest that water stress protects plants from ozone injury mainly through its influence on stomatal aperture rather than through biochemical or anatomical changes.

Plant response to O_3 is controlled by environmental factors, both during plant growth and during exposure. In field studies, O_3 injury was greater on plants grown in moist soil than those grown in drier soils (2, 17, 23) and the injury intensity was proportional to the amount of irrigation water applied (2, 23). Plants used in greenhouse studies exhibited the same type response to water stress as did those in field studies. Plants that were water stressed just prior to O_3 exposure showed little or no foliar injury compared to well-watered plants (5, 8, 11, 12). Only a few days of water stress were sufficient to protect plants from O_3 injury (17, 21). When water stress was eliminated, plants rapidly regained their O_3 sensitivity (2, 21). Stomata of water-stressed plants opened to a smaller degree, closed earlier during the day, and also closed more rapidly in the presence of O_3 (2, 9, 15).

O_3 effects on plants are the result of cellular perturbations. These perturbations are controlled by several factors, including the rate of O_3 uptake through the stomata, scavenging mechanisms which reduce the internal concentration of O_3 or its reaction products, and homeostatic processes which attempt to repair or compensate for the perturbation (20). The resultant injury is a consequence of an interplay among these factors.

The results from field and greenhouse studies cited above suggested that water stress treatments reduced plant response to O_3 probably through partial stomatal closure. However, Heck *et al.* (6) suggested that water stress during plant growth (prior to O_3 exposure) reduced plant sensitivity to O_3 through physiologic changes within the plant, while water stress during exposure reduced plant response through stomatal closure.

Our objective was to determine whether water stress-induced reduction in O_3 sensitivity resulted from short term physiologic alterations within the plant as proposed by Heck *et al.* (6) as well as from reductions in stomatal aperture.

MATERIALS AND METHODS

Plant Culture. Beans (*Phaseolus vulgaris* L. cv 'Pinto 111') were grown from seed in tubular plastic containers (Super cells; volume, 175 cm³; Ray Leach 'Cone-Tainer' Nursery¹, Canby, OR) and watered daily with modified Hoagland solution (21). The plants were grown in a controlled environment chamber on a 16-h light/8-h dark cycle with a photosynthetic photon flux density of approximately 380 $\mu E m^{-2} s^{-1}$ at canopy height. Day and night temperatures averaged 23.5 ± 1 and $18.5 \pm 1^\circ C$, respectively. Six replications of 28 plants each were used for the final data analysis.

Experimental Procedures. When the plants had well-developed root systems (approximately 17 d from seeding) they were transferred into individual membrane water-stress systems (19). The plant root-mass was enclosed in a semipermeable membrane system (Spectrapor 1; exclusion limit, 6,000-8,000 D; Spectrum Medical Industries, Los Angeles, CA) and placed in either a nutrient solution (-35 kPa) or a nutrient solution to which polyethylene glycol (20 M) (mol wt distribution, 14,000-16,000 D) was added to create an osmotic potential of -80 kPa. Although a solution osmotic potential of -80 kPa does not appear to be a large stress, a previous study (21) showed that this level of osmotic stress in the solution caused significant changes in plant indicators of water stress. Over a 3-day period leaf conductance decreased 60%; leaf water potential and top dry weight of bean plants were reduced 13% and 34%, respectively, over a 7-d period. The osmotic potentials of the solutions were controlled at the root-water interface within the semipermeable membrane and thereby controlled plant water potential (19).

After the plants had been maintained in -35 or -80 kPa for 4 days, 12 of the 28 plants in each replicate were sprayed with FC² solutions (10, 15, or 20 μM) until run-off. Previous studies showed that FC induced stomatal opening in both light and dark (4, 22). The FC was dissolved in 0.1 ml ethanol and brought to volume with distilled H₂O. On the morning of day 5, 12 additional plants were sprayed with ABA solutions (100, 250, or 500 μM , four plants per concentration level) and the leaves were allowed to dry (about 1 h). The ABA was dissolved in 0.1 ml of saturated Na₂CO₃ and diluted with a few milliliters of distilled H₂O (pH 7.0). The solutions were brought to volume with 67 mM phosphate buffer (pH 7.0). Two to three drops of Triton X-100 per 50 ml of solution were added as a surfactant to each solution. The remaining four plants served as controls.

Plants were placed in exposure chambers located within growth chambers, equilibrated for 2 h and then exposed to ozone (0 or

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² Abbreviation: FC, fusicoccin.

1.0 $\mu\text{l/l}$) for 2 h (13). The high O₃ concentration was used to clearly demonstrate the plant response to the water stress and chemical (ABA, FC) treatments. O₃ generated by UV light irradiation of air was metered into the exposure chambers to maintain the desired gas-phase concentrations and measured using chemiluminescence analyzers. The O₃ analyzers were calibrated with a transfer standard (10) just prior to each use at the desired concentrations. The transfer standard was calibrated with a dedicated 'UV Standard' operated and calibrated in accordance with the procedures of Paur and McElroy (14). To insure accuracy, both standards (UV and transfer) were subjected to periodic performance audits. Exposures occurred at the same environmental conditions as plant growth.

Leaf resistance measurements were made on half of the plants just prior to exposure with a diffusion porometer on the abaxial surface of primary and first trifoliate leaves. Stress ethylene production and leaf Chl content were used to measure the O₃ response (16, 18). The leaves for ethylene determination were harvested immediately following O₃ exposure. Lateral leaflets of the first trifoliate leaves or primary leaves were excised and placed in a 125-ml flask which were stoppered and incubated in the dark (26°C) for 1.5 h. Thereafter, ethylene concentration was quantified by GC and the leaf area determined (21). The leaves for Chl determination were harvested 72 h after exposure. Chl was extracted from primary leaves and lateral leaflets of first trifoliate leaves in 96% ethanol and the concentration determined spectrophotometrically (24).

The response variables (leaf resistance, ethylene production, and Chl concentration) were first transformed to their respective logarithms prior to statistical analysis to stabilize variances. An analysis of variance was then conducted for each response. The factors included in the data analysis model were the main effects (water stress level, treatment, *i.e.* ABA or FC and O₃), the two-way interactions (water stress by treatment, water stress by O₃, and treatment by O₃), as was the three-way interaction of the main effects in addition to replication. The treatment parameter was modeled as linear function of ABA and FC concentration levels and least squares estimates of the linear response curves were obtained.

RESULTS AND DISCUSSION

Leaf Resistance. The statistical analysis indicated that both the water stress and the treatment (*i.e.* ABA, FC) main effects were very significant (F test, $P < 0.0001$) as was the treatment by water stress interaction ($P = 0.0004$). The analysis showed that both water stress and treatment (ABA, FC) significantly affected leaf resistance. However, the magnitude of the change was not the same for all levels of treatment and water stress as indicated by the significance of the two-way interaction (treatment by water stress). Five d after the plants were placed in the membrane systems, the leaf resistance of trifoliate leaves of control plants grown in the -35 kPa solution (non water-stressed) averaged about 2.5 s cm^{-1} (Fig. 1). Water stress (-80 kPa) increased stomatal resistance about 2-fold to 5.6 s cm^{-1} (Fig. 1), after the 5-d water stress period. Leaf resistance of primary leaves followed the same pattern as the trifoliate leaves (data not shown). Earlier studies with beans using the membrane system produced similar leaf resistance values over a similar time period (21). In those studies, a 2-d water stress treatment of -80 kPa was sufficient to induce stomatal closure increasing leaf resistance about 2.5-fold; this value remained constant for the remainder of the study (5 d).

When the control (-35 kPa) plants were treated with ABA ($500 \mu\text{M}$), the stomatal resistance increased about 70% to 4.2 s cm^{-1} (Fig. 1A). In the water-stressed plants (-80 kPa) treated with ABA ($500 \mu\text{M}$), the stomatal resistance increased 25% to 7 s cm^{-1} . In both water-stressed and non-water-stressed plants,

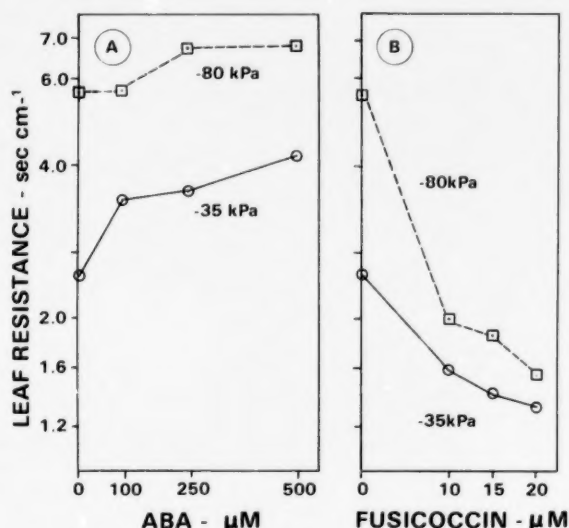


FIG. 1. The influence of ABA and FC on the leaf resistance of trifoliate bean leaves grown at two levels of water stress. Each mean was based on six observations with a standard geometric error of 1.08. The standard geometric error is a measure of the multiplicative variation about the mean. Figure 2A illustrates the ABA response; the ABA was sprayed on the plant 3 h prior to leaf resistance measurements. Figure 2B illustrates the FC response; the FC was sprayed on the plant 27 h prior to leaf resistance measurements. Note that the y axis of the graph is a logarithmic scale.

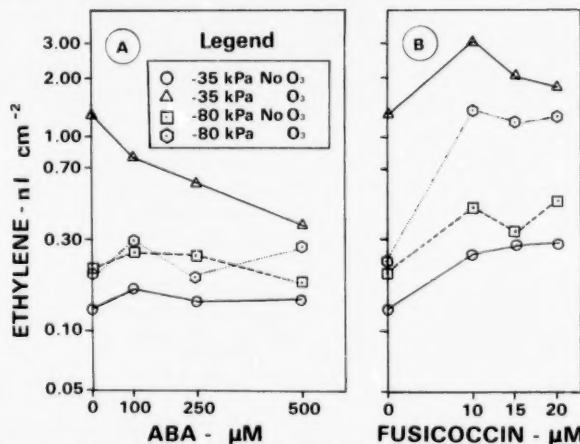


FIG. 2. The interaction of O₃ and plant water status on plant response to O₃. Each mean was based on six observations with a standard geometric error of 1.21. Figure 3, A and B, illustrates the influence of ABA and FC on the O₃ response as determined by stress ethylene production. One leaflet of the first trifoliate was detached immediately following exposure, placed in a flask, and incubated in the dark for 1.5 h prior to measuring the ethylene. Note that the y axis of the graph is a logarithmic scale.

ABA was a significant ($P < 0.05$) factor in inducing stomatal closure. FC, in contrast, induced significant ($P < 0.0001$) stomatal opening in both the -35 and -80 kPa treatments (Fig. 1B). When control plants (-35 kPa) were treated with FC ($20 \mu\text{M}$), the leaf resistance was decreased about 50% to 1.3 s cm^{-1} . In the water-stressed plants (-80 kPa), the FC treatment ($20 \mu\text{M}$) decreased stomatal resistance about 70% to 1.6 s cm^{-1} .

Plant Injury Responses. Stress ethylene production and foliar

Table 1. The Effect of ABA and FC on O₃-Induced Chl Loss

The Chl content is expressed as $\mu\text{g cm}^{-2}$. The plants were water-stressed for 5 d before O₃ treatment. Plants were exposed to either 0 or 1.0 $\mu\text{l/l}$ O₃ for 2 h. Each mean was based on six observations with a standard geometric error of 1.191.

Treatment		0	ABA		Fusicoccin	
			500 μM	% Δ^a	20 μM	% Δ^a
		$\mu\text{g cm}^{-2}$			$\mu\text{g cm}^{-2}$	
-35 kPa	No O ₃	47.3	48.0	+1	36.6	-23
-35 kPa	O ₃	33.3	45.7	+37	39.3	+18
-80 kPa	No O ₃	40.6	37.7	-7	36.0	-11
-80 kPa	O ₃	44.1	44.0	0	32.3	-27

^a % Δ is the per cent change in the Chl content resulting from ABA or FC treatment, respectively.

Chl concentrations were used to monitor the effects of O₃ on the plants (16, 18). All the main effects in the data analysis model had a significant ($P < 0.05$) influence on stress ethylene production; all of the two-way interactions except water stress by treatment (ABA, FC) and the three-way interaction terms were highly significant ($P < 0.0001$). When non-water-stressed plants (-35 kPa) were exposed to O₃, stress ethylene production increased about an order of magnitude immediately following exposure (Fig. 2A). When increasing concentrations of ABA were applied, stress ethylene production decreased significantly in the non-water-stressed plants exposed to O₃, whereas stress ethylene production did not change significantly ($P > 0.10$) in water-stressed plants (-80 kPa) and the non-water-stressed plants not exposed to O₃.

Ethylene production from water-stressed plants (-80 kPa), with or without ABA treatment, was higher than in control plants (-35 kPa) not exposed to O₃, although the differences were not statistically significant ($P > 0.10$). At 500 μM ABA concentration, stress ethylene production from O₃-exposed plants was similar between the plants grown at -35 and -80 kPa, and for water-stressed plants (-80 kPa) and control plants not exposed to O₃ (Fig. 2B). Stress ethylene production from the primary leaves followed the same patterns in response to the various treatments as the trifoliate leaves (data not shown).

FC treatments which induced stomatal opening were associated with significant ($P < 0.001$) increases in ethylene production (Fig. 2B) in all but the non-water-stressed non-O₃-exposed plants ($P = 0.11$). The increased stress ethylene induced by O₃ treatment was much greater than that induced by FC alone in either the water-stressed or non-water-stressed plants. Stress ethylene production in primary leaves followed the same response patterns as the trifoliate leaves (data not shown). Studies with citrus leaf explants showed that FC treatment stimulated ethylene production (3). In this study, FC stimulation of ethylene production was still apparent 1 d after FC treatment. Preliminary studies showed that following FC application, stress ethylene production increased proportionally to the FC concentration (data not shown). Stress ethylene production was increased approximately 15-fold by the 40 μM FC treatment (data not shown).

The effects of O₃ on Chl content were assessed in a similar fashion, but only the control and one ABA (500 μM) and one FC (20 μM) levels were used (Table 1). The O₃ treatment significantly reduced (30%) the Chl concentration in the non-water-stressed plants (-35 kPa). However, in the water-stressed plants (-80 kPa), the O₃ treatment did not significantly decrease the Chl concentration, confirming the response shown with ethylene production; water stress prevented O₃ injury. Treating O₃-sensitive plants (-35 kPa) with ABA prevented the O₃-induced loss of Chl. Treatment with FC caused significant ($P < 0.05$) Chl loss in non-O₃-exposed plants at both water-stress levels, as well as in the O₃-exposed, water-stressed plants. However, in the water-

stressed plants (-80 kPa), the O₃ treatment caused a significantly greater Chl loss than the FC alone. The FC, which induced stomatal opening, rendered the plants as sensitive to O₃ as plants that were not water-stressed.

Leaf resistance was increased and O₃ injury was reduced in plants treated with ABA as previously reported (1, 7). Results from our study confirm findings of earlier studies, *i.e.* that water-stress decreases O₃ injury (2, 12, 17). However, in this study the plants were water-stressed for 5 d prior to exposure, allowing the plants to adapt physiologically to the water-stress conditions before O₃ treatment.

In this study, as in previous ones, conditions that increased stomatal resistance (water stress and ABA) reduced plant response to O₃. Both measures of plant response (stress ethylene production and Chl concentration) yielded the same conclusions; the degree of stomatal opening was more important in controlling the O₃ response than were the physiological changes induced by water stress. It is possible that water stress may induce changes in plant anatomy and thereby influence O₃ sensitivity. However, the water stress period used in this study was not of sufficient duration to permit such growth-dependent changes to occur. In this study, plants that had been water stressed for 5 d were as sensitive as non-water-stressed plants if their stomata were opened chemically, with FC. These data support the concept that the primary means by which water stress protects plants against O₃ injury is through stomatal closure (*e.g.* 1, 15, 21) rather than through physiologic changes within the plant.

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